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Evaluation of Thuringiensin for Control of *Heterodera glycines* on Soybean[1]

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Abstract: The efficacy of ABG-6162A (1.5 L formulation of thuringiensin) for control of *Heterodera glycines* on soybean (*Glycine max* cv. Williams 82) was evaluated in the greenhouse at rates of 0, 10, 100, 500, and 1,000 ppm a.i. and in a field experiment at rates of 0, 3.4, 10.2, and 20.3 g a.i./100 m of row applied in an 18-cm-wide band. In the greenhouse, the 100-ppm rate was as effective as an equivalent rate of aldicarb and caused no phytotoxicity, but the higher rates of thuringiensin were highly phytotoxic. In the field experiment, numbers of females recovered from plots treated with thuringiensin at the 3.4-g and 10.2-g rates were greater ($P \leq 0.05$) than from plots treated with aldicarb at 20.3 g a.i./100 m of row in an 18-cm-wide band or from the *H. glycines*-resistant soybean cv. Fayette. Yield of the untreated Williams 82 control differed significantly only from the 10.2-g rate of thuringiensin, but yield of untreated Fayette was greater than that from any of the treatments involving Williams 82.

Key words: aldicarb, chemical control, *Glycine max, Heterodera glycines,* resistance, soybean, soybean cyst nematode, thuringiensin.

Thuringiensin, the β-exotoxin produced by *Bacillus thuringiensis* Berliner, is active primarily against certain orders of insects (9). It is not a contact poison and must be ingested by the target organism. The toxin inhibits RNA synthesis (8) and insecticidal activity is associated with increased levels of physiological activity between stages of the life cycle, e.g., molting, pupation, and metamorphosis (9). Since the feeding mechanism of plant-parasitic nematodes is different from that of insect pests of plants, it would seem unlikely that thuringiensin would be effective against plant-parasitic nematodes. However, nematodes incubated in solutions containing the toxin were not as infective (4) and exhibited less movement and hatching (6) than those incubated in toxin-free solutions. Use of natural products to control nematodes may be more desirable from an environmental perspective than use of synthetic carbamates and organophosphates. Thuringiensin is less toxic than nematicides currently registered. The oral LD_{50} for the 10 G formulation is 9.4 g/kg (1), compared to 1 mg/kg for aldicarb.

The objectives of this research were to compare the efficacy of thuringiensin (ABG-6162A, Abbott Laboratories, North Chicago, IL) to that of aldicarb and resistant soybean (*Glycine max* (L.) Merr. cv. Fayette) for control of *Heterodera glycines* Ichinohe race 4, under greenhouse and field conditions.

MATERIALS AND METHODS

Greenhouse: The 1.5 L formulation of thuringiensin and the 15 G formulation of aldicarb were evaluated at the rates of 0, 10, 100, 500, and 1,000 ppm a.i. on the *H. glycines*-susceptible soybean cv. Williams 82. The *H. glycines*-resistant Fayette also was included. Infested soil (series Watseka [sandy, mixed, mesic Aquic Hapludolls]; surface layer texture = loamy fine sand, 2.0% organic matter) used for the study was obtained from microplots on the USDA nematology farm at Urbana, Illinois, mixed thoroughly, and found to contain 336 ± 2 cysts and 208 ± 8 second-stage juveniles/200 cm^3 soil.

The soil was apportioned into 1-liter quantities in plastic bags, and the proper amount of each pesticide was added. The soil was mixed thoroughly and 200 cm^3 was added to each of five 250-ml pots. Soil for the two control treatments also was placed

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[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that also may be suitable.

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in bags and mixed. Seeds of Williams 82 and Fayette soybean were germinated in vermiculite. One Williams 82 seedling with a 5-cm-long radicle was transplanted into each pot containing treated soil. In addition, Williams 82 and Fayette seedlings were transplanted individually into pots containing nematicide-free soil. Each of the 10 treatments was replicated five times in a randomized complete block design on a greenhouse bench.

At 30 days after transplanting, phytotoxicity was rated using a 0–4 system where 0 = normal growth, 1 = slight stunting, 2 = moderate stunting, 3 = severe stunting, and 4 = plant death. Plants and soil were removed from the pots, and the number of females was determined by Cobb's gravity sieving technique (2) using nested 850-μm-pore and 180-μm-pore sieves. Roots were placed on the upper sieve and sprayed with a strong stream of water to remove females that adhered to the root system. Approximately 2 liters water was added to the soil from each pot and stirred vigorously; the suspension was sieved to extract females, which were counted with the aid of a dissecting microscope.

Since there were large numbers of missing values due to dead plants and experimental units for which no nematodes were recovered, the PROC GLM procedure of SAS (7) was used to analyze log-transformed data ($\log_{10}$ [x + 1]). Means were separated using Fisher's protected least significant difference (FLSD) at $P \leq 0.05$. Regression analyses were used to compare linear and quadratic effects of thuringiensin and aldicarb.

Field: A site was selected at the USDA nematology farm at Urbana in a field infested with 49–355 *H. glycines* race 4 cysts/250 cm^3 soil. The soil (series Drummer [fine-silty, mixed, mesic Typic Haplaquolls]; surface layer texture = silty clay loam, 6% organic matter, pH 6.0) was maintained at fertility and pH levels recommended by the University of Illinois. The previous crop was Williams 82 soybean. No fall plowing was done and the field received minimum tillage in the spring. The herbicide metolachlor was preplant incorporated at the rate of 2.78 kg a.i./ha.

Treatments were arranged in a randomized complete block design with four replications. Each experimental unit consisted of four 7-m-long rows on 76-cm centers. The six treatments included the susceptible cultivar Williams 82 and the resistant cultivar Fayette as untreated controls and four pesticide treatments planted with Williams 82. Thuringiensin was applied immediately before planting at the rates of 3.4, 10.2, and 20.3 g a.i./100 m of row in an 18-cm-wide band and aldicarb was applied at the rate of 20.3 g a.i. Both pesticides were incorporated 5 cm deep into the soil with a rototiller. Thuringiensin was applied with a four-row CO_2-pressurized backpack sprayer at 7.03×10^{-2} kg/cm^2 pressure and 748 liters/ha delivery rate through four Delvan (West Des Moines, IA) LE3 80° nozzles. Since the viscocity of the high rate of thuringiensin might have interfered with accurate delivery, the pesticide was diluted with equal parts of water and applied twice. Aldicarb was applied with a Precision Machine (Precision Machine, Lincoln, NE) single-row applicator equipped with a Noble (Sac City, IA) metering device. Seeds were planted at the rate of 33/m with a tractor-drawn two-row planter on 4 June 1985.

Numbers of cysts and females were determined at planting and 6 weeks after planting, respectively. Twenty cores were collected with a 2-cm-d soil probe in a zigzag pattern from the two center rows of each plot 3–7 cm from the base of plants and to a depth of 15 cm. Nematodes were extracted from 250-cm^3 aliquants by Cobb's gravity sieving technique (2) with nested 850-μm-pore and 180-μm-pore sieves and counted with the aid of a dissecting microscope. The efficacy of the treatments in controlling *H. glycines* was determined by comparing female counts and by calulating a developmental factor (Df = females at 6 weeks/gravid cysts at planting).

A two-row combine was used to harvest 4.7 m from the two center rows of each

TABLE 1. Numbers of first generation females of *Heterodera glycines* and phytotoxicity to soybean 6 weeks after initiation of a greenhouse experiment to evaluate control of *H. glycines* with thuringiensin and aldicarb.

Treatment (a.i. ppm)	Females†	Phytotoxicity‡
Control (Williams 82)	310§	1.0
Thuringiensin (10)	293§	0.8
Thuringiensin (100)	30‖	0.8
Thuringiensin (500)	0‖§	3.0
Thuringiensin (1,000)	0‖§	3.4
Aldicarb (10)	136‖§	0.3
Aldicarb (100)	23‖	1.0
Aldicarb (500)	0‖§	1.2
Aldicarb (1,000)	0‖§	2.4
Control (Fayette)	31‖	0.3
CV %	15.3	

One control and all nematicide treatments were planted with susceptible Williams 82 soybean. A second control was planted with the resistant cv. Fayette.

† Number of females per pot containing 200 cm^3 soil.

‡ Based on stunting where 0 = none, 1 = slight, 2 = moderate, 3 = severe, and 4 = dead plant. Data were nonparametric and were not analyzed.

§ Differs from the Fayette control at $P \leq 0.05$ using transformed data ($\log_{10}[x + 1]$).

‖ Differs from the Williams 82 control at $P \leq 0.05$ using transformed data ($\log_{10}[x + 1]$).

plot. Seeds were cleaned, dried, and weighed. Moisture content was adjusted to 14% and yields were determined.

Data were subjected to analysis of variance and means were separated using Fisher's protected LSD ($P \leq 0.05$). Correlation coefficients were determined between yield and numbers of females and between yield and Df.

RESULTS

Greenhouse: Thuringiensin at 100 ppm and aldicarb at 100 ppm were as effective as Fayette in reducing numbers of females when compared with the Williams 82 control (Table 1). There was minimal phytotoxicity associated with these treatments. Aldicarb at 500 ppm also was effective in reducing numbers of females, but this treatment resulted in slight to moderate phytotoxicity. No females were recovered from thuringiensin at 500 or 1,000 ppm, and severe phytotoxicity was associated with these two treatments. Thuringiensin at 10 ppm and aldicarb at 10 ppm were not as effective as the other treatments in reducing the number of females, but aldicarb at 10 ppm was more effective than thuringiensin at this rate. Minimal phytotoxicity was observed with these two treatments. Slight stunting was observed for the Williams 82 control. The regression equations relating numbers of females (y) to treatment rate (x) were $y = 2.239x - 0.0046$ ($r^2 = 0.94$, $P \leq 0.001$) for the effect of thuringiensin rate and $y = 1.918x - 0.0038$ ($r^2 = 0.94$, $P \leq 0.001$) for the effect of aldicarb rate. There were no significant differences ($P \leq 0.05$) either between intercepts or between slopes of these two regression equations.

TABLE 2. Effect of aldicarb, *Heterodera glycines*-resistant Fayette soybean, and thuringiensen on *H. glycines* first generation population development and yield of soybean at Urbana, Illinois.

Treatment and rate (g a.i./100 m of row)	Yield (kg/ha)	Females (no./250 cm^3 soil)	Df†
Control (Williams 82)	1,597	47	0.44
Aldicarb (20.3)	1,940	2	0.02
Thuringiensin (3.4)	1,743	38	0.36
Thuringiensin (10.2)	1,886	23	0.34
Thuringiensin (20.3)	1,764	16	0.18
Control (Fayette)	2,680	1	0.01
FLSD ($P \leq 0.05$)	222	17	0.24
CV %	7.9	53.4	72.1

One control and all nematicide treatments were planted with Williams 82 soybean. A second control was planted with the resistant cv. Fayette.

† Df = females at 6 weeks/gravid cysts at planting.

Field: Yield of Fayette was superior to all other treatments (Table 2). Yields from plots with the three rates of thuringiensin were not significantly different from the aldicarb treatment, and the lowest yield was obtained from the untreated Williams 82. No phytotoxicity was observed. Fewer females were recovered from the Fayette and aldicarb treatments than from the 3.4-g and 10.2-g rates of thuringiensin and the Williams 82 control. Numbers of females in plots with the 20.3-g rate of thuringiensin did not differ ($P \leq 0.05$) from those in the Fayette and aldicarb treatments. Similarly, Df for the Fayette and aldicarb treatments differed from the 3.4-g and 10.2-g rates of thuringiensin but not from thuringiensin applied at 20.3 g a.i. The correla-

tions between yield and numbers of females and between yield and Df were −0.64 and −0.62 ($P = 0.001$), respectively.

Discussion

The efficacy of thuringiensin in controlling *H. glycines* under greenhouse conditions was similar to that of aldicarb and Fayette. Under field conditions, the reduction in numbers of females resulting from treatment with thuringiensin was not as striking as in the greenhouse test, and the yield increase was not as great as that from treatment with aldicarb. Neither thuringiensin nor aldicarb provided control equal to that of the resistant cultivar Fayette. Thuringiensin has potential for use in control of *H. glycines*. Higher rates of thuringiensin would be required, and the 1.5 L formulation would be impractical because of the large volumes of liquid necessary for application. Although phytotoxicity was not observed in the field, the greenhouse study showed that phytotoxicity could be a concern.

The mode of action of thuringiensin in insects involves prevention of RNA synthesis (8), but the mode of action in *H. glycines* and the life stage of the nematode affected by thuringiensin are not known. It is possible that the activity was not due to thuringiensin but rather to another constituent of the formulated product; however, previous studies (4,7) that demonstrated deleterious effects of thuringiensin on infectivity, hatch, and survival of *Meloidogyne* spp. involved use of toxin obtained from cultures, not formulated material. In field soil, populations of *Meloidogyne* were lower at a site infested with *B. thuringiensis* and higher at a site from which the bacterium was not isolated (5).

In contrast to possible groundwater contamination from various synthetic, soil-applied nematicides, some natural products may not pose significant environmental dangers. In attempts to control insects, recombinant DNA technology has been used to transfer toxin genes from *B. thuringiensis* into epiphytic bacteria and to produce transgenic plants (3). Development of nematicides from natural products, especially those with potential for genetic engineering, may provide a means of alleviating environmental risks.

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